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The *architecture* of the retromer coat.

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Retromer is a master regulator of endosomal cargo sorting. Using cryo-EM, Kovtun, Leneva and colleagues have now revealed how, in yeast, this multi-protein complex is assembled on tubular membranes to form a coat complex that orchestrates the process of tubular-based cargo sorting.

The endosomal network orchestrates the intracellular sorting of thousands of integral membrane proteins (together termed 'cargo') between two fate decisions: cargo is either transported to the lysosome for degradation or is retrieved from this fate for onward transport to organelles that include the cell surface and the biosynthetic pathway (Cullen and Steinberg, 2018). While a detailed understanding of how cargo is sorted for lysosomal degradation has emerged, those molecular events that define how cargo, such as signalling receptors, nutrient transporters, ion channels, adhesion molecules and polarity cues undergo retrieval remain poorly described. Central to the current view of cargo retrieval, is the observation, made over 30 years ago, that cargos undergoing the retrieval process are sorted into endosome-associated tubular profiles that undergo scission to form isolated cargo-enriched transport carriers (Cullen and Steinberg, 2018). The field has focused therefore on defining how these endosomal tubules are generated and how their biogenesis is co-ordinated with the sorting of cargo proteins that are to undergo retrieval. In a recent study, Kovtun, Leneva and colleagues (Kovtun et al., 2018) have provided new insight into these questions by revealing how one master regulator of endosomal cargo retrieval, the multi-protein retromer complex, undergoes assembly on tubular profiles to form a retrieval coat complex.

The yeast retromer is a pentameric assembly of a heterotrimer of Vps26, Vps35 and Vps29, and a heterodimer of Vps5 and Vps17 (Seaman et al., 1998): Vps5 and Vps17 are phospholipid-binding Bin/Amphiphysin/Rvs (BAR)-domain containing sorting nexins (SNX-BARs) that display an inherent ability to drive membrane tubule formation (Carlton et al., 2004; van Weering et al., 2012). Structural studies of individual retromer components and partially assembled retromer sub-complexes have been reported (Collins et al., 2005; Hierro et al., 2007; van Weering et al. 2012; Lucas et al., 2016), but the major breakthrough made

by Kovtun and colleagues has been to visualise the assembly of the retromer in the context of a membrane tubule. To achieve this, the authors assembled from the fungal species *Chaetomium thermophilum* a retromer composed of Vps26, Vps29 and Vps35 bound to a homodimer of Vps5. When added to liposomes, retromer (in a Vps5-dependent manner) induced the formation of membrane tubules decorated with a protein coat approximately 15 nm thick. Cryo-electron tomography and reference-free subtomogram averaging revealed the structural arrangement of the coat, alignment of which provided density maps into which could be unambiguously modelled known retromer structures thereby revealed a pseudo-atomic structure of the assembled retromer coat (Kovtun et al., 2018).

The coat is composed of an inner shell of the membrane-associated Vps5 homodimers that are orientated to allow their PX (phox homology) domains and the positively-charged ends of their BAR domains to interact with membrane phospholipids (Figure 1A). Similar to other BAR domain-containing proteins, the Vps5 dimers assemble into an oligomeric helical array via tip-to-tip contacts between the BAR domains of consecutive molecules, while lateral contacts are made between the PX domain of adjacent Vps5 homodimers. In the outer shell, Vps26 dimers are directly docked on the Vps5 proteinaceous lattice and provide the sole contacts with the inner shell. Importantly, the Vps26 dimers contact four Vps5 dimers hence stabilizing the underlying helical assembly through bridging two neighbouring rows of Vps5: this is consistent with Vps26:Vps35:Vps29 being able to promote the tubulating activity of the Vps5:Vps17 heterodimer (Purushothaman and Ungermann, 2018). Each Vps26 molecule interacts with the amino-terminus of Vps35. The extended α -solenoid structure of Vps35 (Hierro et al., 2007) serves to present the carboxy-terminus to form a dimer interface with the carboxy-terminus of a neighbouring Vps35 molecule (Vps29 binds to Vps35 on the opposite surface of this dimer interface). This leads to the formation of a series of extended arch-like structures that point away from the membrane surface. Interestingly, the most prevalent retromer mutation associated with familial forms of Parkinson disease, VPS35(p.D620N), maps to a region adjacent to this interface suggesting that, in addition to its known effects on association to the actin-polymerising WASH complex (Cullen and Steinberg, 2018), this mutation may subtly perturb dimer formation and retromer coat assembly (Kovtun et al., 2018). Finally, the Vps5 helical array is irregular (pseudo-helical) and Vps26 dimers can dock at six relative positions, suggesting a high degree of coat plasticity. This may provide flexibility for nestling into the coat of not only cargo proteins but also retromer accessory proteins such as the Rab GTPase Ypt7 (Liu et al., 2012) and the sorting nexin Snx3 (Strochlic et al., 2007), some of which are known to promote retromer-mediated tubule formation (Purushothaman et al., 2017; Purushothaman and Ungermann, 2018). Kovtun and colleagues do go some way to addressing these issues by confirming the

presence of a similar retromer coat *in vivo* through analysing a dataset of cryo-electron tomograms of endosomal tubules from the green alga *Chlamydomonas reinhardtii*, tubules that presumably are enriched in endogenous cargos and accessory proteins. Further detailed *in vitro* reconstitutions of retromer coats are certain to be required to define the influence that cargo and accessory proteins may have on the architecture of the yeast retromer coat.

Does the resolved yeast retromer coat help our understanding of retromer function in higher metazoans? Unlike the situation in yeast the mammalian VPS26:VPS35:VPS29 and those SNX-BARs that correspond to yeast Vps5 and Vps17 do not appear to form a stable protein complex (Norwood et al., 2011) and moreover, the two complexes can display separate functional roles (Kvainickas et al., 2017; Simonetti et al., 2017). The yeast pentameric retromer may therefore be the exception rather than the rule. Indeed, this is reflected in the metazoan nomenclature, where ‘retromer’ is used to refer to just the VPS26:VPS35:VPS29 heterotrimer (a nomenclature that we will use from here). Further complexity comes from evidence that metazoan retromer also associates with the equivalent of yeast Snx3, SNX3 and a protein not expressed by yeast, sorting nexin-27 (SNX27) to form functionally distinct complexes: these sorting nexins lack BAR domains and hence do not drive tubule formation (Harterink et al., 2011; Temkin et al., 2011; Steinberg et al., 2013). For SNX3 the association with retromer exposes a binding site at the interface between SNX3 and VPS26 for cargo containing a \emptyset x(L/M/V) sequence motif (where \emptyset is a bulky aromatic residue) (Lucas et al., 2016) (Figure 1A). In the yeast retromer coat this cargo-binding interface would be occluded by an α -helix from Vps5, suggesting that the interaction with cargo-bound SNX3-retromer and an equivalent yeast retromer coat would be mutually exclusive. For SNX27, which regulates the retrieval of >400 cargos that contain a specific type of carboxy-terminal PDZ ligand (Cullen and Steinberg, 2018), it associates with retromer through a groove in VPS26 that, in the tubular yeast retromer coat, would be accessible for binding (Gallon et al., 2014) (Figure 1A). Whether, in an equivalent coat assembly, the other domains of SNX27 would sterically clash to preclude simultaneous binding, remains to be established.

Importantly, both SNX3 and SNX27 are able to directly associate with the membranes in part through their phosphoinositide-binding PX domains. It may be therefore that cargo associated SNX3-retromer and SNX27-retromer complexes directly assemble on the membranes to form coats that are different (perhaps assembled on ‘flat’ membranes) but nonetheless related (perhaps retaining the retromer arches) to those described by the tubular yeast retromer coat (Figure 1B). Future efforts will need to define how these different metazoan SNX-retromer complexes assemble on membranes, whether separate SNX-retromer coats organise the formation of different cargo-enriched carriers, and whether

different SNX-retromer complexes may sequentially hand off their cargos to one another as part of a processive pathway. It will also be interesting to explore if the recently identified retriever, which shares striking predicted structural similarity to retromer (McNally et al., 2017), could form similar retrieval coats on membranes. The amazing power of cryo-electron tomography is certain to provide continual exciting insights into those coat complexes that underlie endosomal cargo retrieval.

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Figure 1. Overview of retromer assembly on endosomes.

(A) Proposed model of retromer assembly on endosomes in yeast (left panel - as described by Kovtun, Leneva and colleagues (PDB:6H7W)) and in higher metazoans (right) cells through the proposed model for how the SNX3-retromer and SNX27-retromer complexes could assemble on the endosomal membranes. The SNX3-retromer assembly corresponds to the DMT1-II:SNX3:VPS26:VPS35 structure described by Lucas et al., (PDB:5F0L). The assembly of the SNX27-retromer complex is a highly speculative model generated as follows. The retromer structure (VPS26:VPS35, PDB:5F0L) was superimposed on the crystal structure of the SNX27 PDZ domain bound to VPS26 described by Gallon et al., 2014 (PDB:4P2A). The PX domain of SNX27 (PDB:4HAS) was superimposed on the PX domain of SNX3 as suggested by Lucas et al. 2016 and linked to the crystal structure of the FERM-like domain of SNX17 described by Ghai et al., 2013 (PDB:4GXB). The blue dashed lines represent linker segment between the domains of SNX27, which could however be packed into a more globular structure. The dashed brown line represents the cytosolic tails of cargo proteins containing the sorting motifs that bind to SNX3 or SMX27. (B) Proposed model for the assembly of SNX-retromer complexes in mammalian cells. The retromer senses and captures cargo, either directly or through the interaction with SNX3 and SNX27. Increased cargo concentration may lead to dimerization of SNX3-retromer and SNX27-retromer complexes into arched dimers, similarly to that observed in the yeast system by Kovtun, Leneva and colleagues. The WASH complex subunit FAM21 binds and clusters multiple retromer dimers hence coordinating the retrieval of cargo with the organisation of a retrieval subdomain (Jia et al., 2012). Finally, the crowding of cargo-bound retromer dimers could induce subtle membrane deformation thereby aiding the recruitment of the SNX-BAR proteins for the generation of recycling tubules.

The complete model of the retromer coat in the top left panel of Figure 1A was obtained from Supplementary material Video 4 from Kovtun, O., Leneva, N., Bykov, Y.S., Ariotti, N., Teasdale, R.D., Schaffer, M., Engel, B.D., Owen, D.J., Briggs, J.A.G. & Collins, B.M. Structure of the membrane-assembled retromer coat determined by cryo-electron tomography. Nature doi: 10.1038/s41586-018-0526-z (2018).

